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13. ABSTRACT *(Maximum 200 words)*

An interdisciplinary group of scientists was assembled to examine heat detection by biological systems with the goal of prototyping physical sensors based on the unique mechanisms found in nature. The focus of this research was heat detection systems of snakes and beetles. In conclusion, our evidence strongly supports the hypotheses that receptors in the pit organ of pit vipers are temperature receptors that are spectrally tuned based on the absorptive properties of the surrounding tissue and suggest that unique receptors with high sensitivity to low temperature stimulus are present in the pit membrane enabling this organ to exhibit a highly sensitive and unique response to thermal stimulus. Work is in progress in our laboratory to further elucidate the molecular and cellular basis of heating sensing capability of pit organ.

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Biological Detection Systems for Electromagnetic Spectral Signatures

Final Report for
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Department of the Air Force
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Bolling AFB, DC 20332-0001

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May, 2003

Final Report
F49620-98-1-0480

Title: Biological Detection Systems for Electromagnetic Spectral Signatures
PI: A.J. Welch

Executive Summary: An interdisciplinary group of scientists was assembled to examine heat detection by biological systems with the goal of prototyping physical sensors based on the unique mechanisms found in nature. The focus of this research was heat detection systems of snakes and beetles.

Research in the initial phase clearly defined irradiance thresholds [W/cm^2] for pit vipers and *Melanophila*. Electrophysiological measurements as a function of wavelength from 400 nm to 10 μm demonstrated that the threshold irradiance of the pit viper was directly related to membrane absorption for water and hemoglobin in the pit membrane. The pit viper responded to heat generation rather than direct absorption to photons at a particular wavelength. Once the detection threshold was measured it was possible to use the physical shape of the pit to calculate maximum separation distances between a black body target and detector threshold. Heating of the pit membrane by photon absorption caused variations in perfusions which modulated nerve activity. Based on electrophysiological data and thermal modeling, we believe that an important aspect of the snake response is the change in temperature with time (dT/dt).

Wavelength dependent IR thresholds of neural activity in the *Melanophila* were reported as 50% probability detection thresholds. The irradiance threshold at 3.39 μm was used to calculate the distance between a forest fire and the beetle. Calculations show that at this irradiance threshold, the beetle could detect an average forest fire at a distance no greater than ~1km. Numerical modeling results agreed with the experiments and confirmed that the pit membrane is brometric rather than photonic.

A wavelength selective infrared microbolometer was fabricated and tested. This demonstrated the feasibility of integrating 'color disorientation' into room temperature infrared detectors. IR detection in the beetle involved photon absorption in its 15 μm diameter sensilla and thermal expansion of the sensilla. Optical measurements of the sensilla during IR irradiation produced proof of nm surface displacement. Thresholds were defined and classes of mechanisms identified.

In conclusion, our evidence strongly supports the hypotheses that receptors in the pit organ of pit vipers are temperature receptors that are spectrally tuned based on the absorptive properties of the surrounding tissue and suggest that unique receptors with high sensitivity to low temperature stimulus are present in the pit membrane enabling this organ to exhibit a highly sensitive and unique response to thermal stimulus. Work is in progress in our laboratory to further elucidate the molecular and cellular basis of heating sensing capability of pit organ.

Introduction: A consortium composed of engineering and science laboratories at five institutions of higher learning collaborated to investigate and create thermal sensors to mimic novel methods for IR detection achieved in nature. The group included:

University of Texas at Austin (UTA)
  Biomedical Engineering
    (Drs. Pearce, Rylander and Welch)
  Electrical and Computer Engineering
    (Dr. Neikirk)
  Biochemistry and Molecular Biology
    (Dr. McDevitt)
University of Texas Medical Branch (UTMB)
  Biomedical Engineering
    (Dr. Motamedi)
Texas A&M University (TAMU)
  Meical Biochemistry and Genetics
    (Dr. Bayley)
Florida Institute of Technology (FIT)
  Biology
    (Dr. Grace)
Iowa State University
  Material Science and Engineering
    (Dr. Tsukruk)

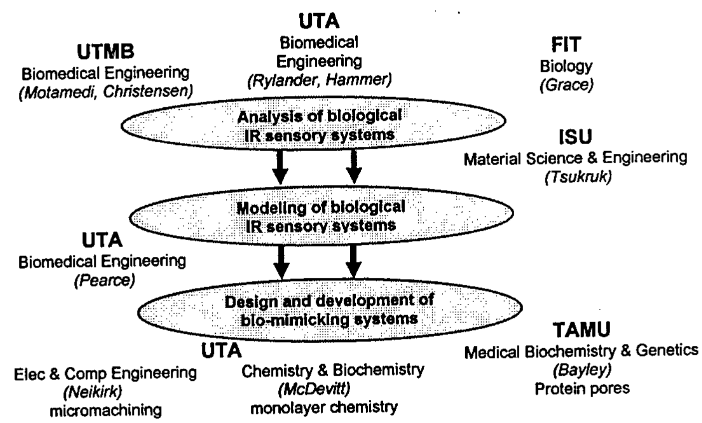

Figure 1.

This research brought together a truly interdisciplinary group with the skills to delineate the IR sensory systems of biological systems and create physical systems to measure and process IR signatures. All participants were actively involved in graduate education. The research provided a wide range of science and engineering tasks that fostered interdisciplinary training of students and broadening of the horizons of the participating faculty.

The specific goals for this cooperative program were (1) the measurement and analysis of infrared sensory systems of pit vipers and *Melanophila acuminata* (Jewel beetle); (2) sensor transduction, amplification, noise reduction and image (data) processing; (3) sensor and transduction modeling; and (4) design and development of a bio-mimicking system.

Project Summaries

Publications (all collaborators)	**Total**
Refereed scientific/technical journals	44
Peer scientific/technical journals	11
Generic scientific/technical journals	8
Generic magazines	2
Scientific/technical books	8
Other book	0
Invited society-level presentations	35
Invited society-level session chair	10
Other society-level presentations	20

Other invited presentations, conferences, sessions, etc.	7
Other presentations, conferences, sessions, etc.	5
Patents and patent disclosures	1
Unpatented inventions	0
Other (identify) PhD/MS	3/3

Management: The research was divided into three major tasks (see Figure1). The first identified the structure of biological infrared sensors through physical measurements (UTMB, ISU, UTA) and electrophysiology (UTMB, FIT, UTA). Dr. Grace's experience with snake electrophysiology was integrated with precision stimulus and instrumentation provided by Dr. Pearce (UTA), neurophysiology and neuroelectricity by H.G. Rylander, MD (UTA) and sensor physiology by Dr. Christensen (UTMB).

The second task was to model the optical (Motamedi-UTMA, Hammer-UTA) and thermal response (Pearce-UTA) of these biological systems. The third task was to support three paths of IR sensor development: (1) protein pores (Bayley-TAMU); (2) monolayer chemistry (McDevitt-UTA); and (3) micromachining (Neikirk-UTA).

Background. Nature provides IR detection systems that are reported to be superior to current technologies which require cryogenic cooling to achieve their high degree of selectivity and sensitivity. Animals use microscopic IR detection systems for basic survival, without the cumbersome requirements of man-made materials. Examples of extraordinary photothermal (certain snakes) and photomechanical (beetles) infrared sensory systems can be found in nature.

Anatomy and physiology: Infrared sensory system of snakes

Highly sensitive imaging forming infrared systems exist in two families of snakes, the Boidae and the Viperidae. These infrared-detecting systems are superior to all artificial and all known biological infrared detectors, as they combine 0.003¡C thermal resolution, microscopic size, and self-repair in an uncooled system. In the former group, the sensor comes in the form of several heat receptors that line the supra- and infralabials. The receptors may be intraepidermal (e.g., Boa constructor) or within shallow grooves (e.g., Python reticulatus). Most pythons and many boas also have facial pits, which form an array around the rim of the mouth in or between labial scales. Other boid snakes are also infrared-sensitive, but simply lack the elaborate pit organ.

In the Viperidae family, the receptors occur in pit vipers (Crotalinae) but not in common vipers (Viperinae). In the Crotaline snakes, there are two pits (hence their name) on either side of the head between the eyes and nostril. Within the pit, there are two cavities separated by a pit membrane which contains the receptor. The membrane consists of a central layer of nerve fibers and vasculature surrounded by an outer and inner epithelial layer. Both layers are cornified and are shed with the rest of the skin, the inner epithelial layer discarded through a small channel from the inner cavity to the exterior. The entire thickness of the membrane varies from ~15 μm in the center to ~20 μm where it attaches to the cavity walls.

Both the infrared and visual systems (along with chemical and tactile cues) are involved in prey detection by boids and pit vipers, but prey detection and localization do not require the eyes. A congenitally blind rattlesnake can accurately strike prey with an efficiency and precision comparable to sighted animals of the same species [Kardong and Mackessey, 1991].

Early behavioral and electrophysiological studies determined that the pit organs allow the snakes to detect warm objects. The mechanism by which infrared radiation is converted to nerve impulses is not known. Recent work demonstrating the organ's sensitivity to conducted heat (flowing water) [DeCock Buning, *et al.*, 1981a] and other forms of radiation [Harris and Gramow, 1971] has indicated that the sensor may function as an energy detector.

The boid infrared-receptive organ consists of a mass of IR-sensitive nerve terminals just under the epidermis of the skin. The fibers that form these terminals originate from cells in the trigeminal ganglion, which in all vertebrates, subserves the senses of pressure, pain, and temperature in the face. IR information conveyed to these trigeminal neurons is sent on to the brain stem, and then to higher brain centers through multi-synaptic connections. The projections of IR-sensitive neurons are arranged to produce a spatial representation within the brain of the snake's infrared environment. This spatial mapping is analogous to that produced by the visual projections to the brain, and in fact, these two sensory modalities merge in the optic tectum of the snake's brain. Thus, infrared-sensitive snakes ÒseeÓ both visible light and infrared radiation, and these two modalities operate in precise spatial register within the brain [Hartline, Kass, and Loop, 1978].

There have been many studies of snake infrared sensors in the previous thirty years, 1992. Most have dealt with the anatomy and physiology of the sensory system, particularly the electrophysiology [Goris and Terashima, 1976] and neurophysiology [Kishida *et al.*, 1982], as well as behavior [DeCock Buning *et al.*, 1981b].

While in the last few decades significant attention has focused upon the neuroanatomy of snake IR-sensing systems, absolutely nothing is known about the mechanisms involved in converting an IR stimulus into an electrochemical neural signal. Grace has undertaken a detailed analysis of the transduction mechanism operating in the IR receptor terminal. These receptor terminals are massed within the membranes of easily recognized facial pits, and are easily removed for study. IR-sensitive neuronal terminals within the pit organ are large spherical structures, the most characteristic feature of which is the abundance of mitochondria contained within them. The fact that most of the cytoplasmic space is filled with mitochondria suggests that they play a fundamental role in IR signal transduction. One report has suggested that they play a primary role in signaling because they change configuration in response to infrared stimulation. Experiments using physiologically-relevant stimuli in Dr. Grace's laboratory, however, indicate that these earlier observations are an artifact produced by high intensity laser stimulation of the pit organ. Even so, the abundance of mitochondria indicates that they either play a primary role in signaling, or that they perform some secondary function such as providing energy for the biochemical signaling machinery. If mitochondria are a fundamental component of IR signaling, they may have unique biochemical or functional properties. Likewise, the terminal as a whole may have a biochemical makeup unique among sensory nerve endings.

The spectral sensitivity of the pit organ is also unknown. Previous experiments have employed a variety of stimulating devices including the human hand [Terashima and Goris, 1975], visible light sources [Terashima, Goris and Katsuki, 1968], and lasers of various wavelengths and intensities [DeCock Buning, Terashima, and Goris, 1981a, b; Harris and Gamow, 1971]. The intensities of many of these sources were far above anything normally encountered by this sensory system. There has been no systematic effort made to determine either the spectral sensitivity of the pit organ or the spectral transmission of the tissues overlying the receptor terminals. Dr. Grace's observations indicate that the surface of the pit organ has a structure unique to the pit and the pit surface may act as a spectral filter or an anti-reflective coating.

The infrared-sensitive pit organ is a high specialized and well-organized cellular infrared detector, and the surface of the pit organ has a very unusual arrangement. The highly ordered arrangement of the pit organ surface suggests that it may act as a spectral filter, functioning like a grating monochromator. Alternatively, this surface structure which overlies the receptor terminals may function as an anti-reflective coating. In addition, Grace has shown that the pit organ has unusual spectral absorption/reflectance properties; the pit organ may be ÒtunedÓ to a particular region of the electromagnetic spectrum, that between 8 and 12 μm wavelength.

Infrared sensory system of the jewel beetle

A thoracic pit organ has recently been discovered in the beetle of genus *Melanophila* [Schmitz *et al.*, 1997]. The organ functions to locate forest fires because the beetle larvae only survive in freshly burnt wood. The insects are capable of detecting forest fires up to a distance of 50 km. Although they may have a similar function, namely the detection of infrared radiation, the pit organs of the snake and beetle differ immensely in structure and mechanism. Whereas the sensor element in the snake pit is a single membrane that contains nerve fibers innervated by the trigeminal nerve, the sensor element of the beetle pit is the sensilla. There are between 50 and 100 sensilla at the bottom of each pit. The dome-shaped sensilla is shown in Figure 1. Each sensillum consists of a sphere suspended from a cuticular dome. The sphere is innervated by a single sensory neuron through a tubular dendritic tip. Schmitz *et al.* suggest that the sensor functions as a mechanoreceptor, by responding to infrared radiation with deformation of the dendritic tip.

Infrared radiation of only 0.06 mW/cm^2 in the 2.5 μm to 4.0 μm causes the antennae of non-flying *Melanophila* to twitch. This wavelength band corresponds to the emission maximum of a forest fire [Evans, 1966]. Schmitz *et al.* demonstrated through the electrical activity measured from single units of individual sensilla of the pit organs that these organs functioned as infrared sensors.

Also, experiments by Schmitz *et al.* showed that the infrared receptors responded to mechanical deformation. Schmitz *et al.* note that, ÒThe spheres of the pit organ consist of organic molecules (endocuticle) which have C-H and O-H groups. Such groups have stretch resonances near 3 μm and thus absorb strongly near this wavelength.Ó

Infrared energy entering the pit is absorbed. The resulting heat source [W/m^3] increases the volume of the spheres (see figure 2) and introduces a deformation of the dentritic tip. Schmitz *et al.* estimate that infrared radiation of a few mW/cm^2 causes deformation of the membrane. They hypothesize that a 1 nm displacement is sufficient to

produce a response in the beetle mechanoreceptors. Thus the beetle IR system is similar to the mechanical action in photoacoustic spectroscopy.

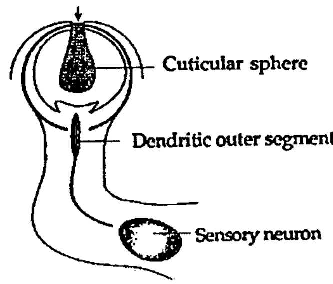

Figure 2. Diagram of the infrared pit organ of Buprestid beetles (adapted from Schmitz *et al.*, 1997)

Summary of Research Accomplishments

A. Infrared detection in *Melanophila acuminate.* Drs. Welch, Rylander and Hammer:

1. Publications

Drs. Welch, Rylander, Hammer Publications	Total
Refereed scientific/technical journals	12
Peer scientific/technical journals	6
Generic scientific/technical journals	1
Generic magazines	0
Scientific/technical books	1
Other book	0
Invited society-level presentations	2
Invited society-level session chair	1
Other society-level presentations	6
Other invited presentations, conferences, sessions, etc.	0
Other presentations, conferences, sessions, etc.	0
Patents and patent disclosures	0
Unpatented inventions	0
Other (identify) PhD/MS	1/1

2. Scientific and/or technical accomplishments
   a. Infrared Detection in *Melanophila acuminata*
      (i) Accomplishment:
      Electro-physiological measurements were made of neural activity as a function of IR irradiance [W/cm^2], wavelength, and irradiance duration.

      Significance:
      Probit analysis to determine irradiance thresholds provided evidence that the *Melanophila*'s LD-50 IR detection limit of a 10-hectare at 700°C is ~1.4 km. Considerable electro-physiology data including flicker fusion results were added to the scientific literature.

      (ii) Accomplishment:
      Optical Coherence Reflectrometry was used to measure pit organ displacement as a function of irradiance [W/cm^2] and irradiance duration. The resulting thermal expansion was modeled as a one dimensional heat conduction and material expansion problem.

      Significance:
      Thermal expansion of the sensilla (*Melanophila* IR detector organ) provided information for hypothesizing that the 15 μm round sensilla not only expands when heated but may rotate about its distal attachment. This data supported

the assumption of a mechanical receptor as the conversion process between movement and electrical activity.

3. Scientific and technology transitions
   a. Infrared detection in *Melanophila acuminata*
      (i) Locating *Melanophila* larvae
      Dr. Apel (Germany), assisted Dr. Rylander (UT) locate *Melanophila* larvae for use in Austin

      (ii) Dr. H. Schmitz (University of Bonn) came to Austin to work with Drs. Hammer and Rylander (UT) on instrumentation and procedures for electrophysiological measurements in the *Melanophila*.

      (iii)Dan Hammer worked with Coherent, Inc. to establish a tunable IR (1-10μm) stimulus system.

      (iv)Dr. Tom Milner (UT) helped Dan Hammer design and build instrumentation for thermal expansion measurements.

      (v) Dan Hammer (UT) shared all electrophysiological results with Dr. H. Schmitz, (University of Bonn)

      (vi)Dr. Hammer's measurements of optical properties, electron microscopic pictures of sensilla, and thermal expansion was shared with Dr. Dean Neikirk (UT) for sensor design and Drs. Pearce (UT), N. Wright (U Maryland, Baltimore County) and Lai (OU) for thermal modeling.

      (vii) Dr. Hammer provided Dr. Stone (USAF) and V. Tsukruk (ISU) with *Melanophila* for measurements of physical and thermal properties.

      (viii) Dr. Hammer assisted Dr. Neikirk experimentally evaluate the IR selectivity of machined microbolometer.

4. Other

   - The dissertation and papers of Dan Hammer provide excellent descriptions of the extent of technology and results provided with the IR detection in *Melanophila.*
   - *Melanolphila* featured in *Science News* March 3, 2001, Vol. 159, no. 9, pp 140-141.

Hammer DX, H Schmitz, A Schmitz, HG Rylander III, AJ Welch, "Sensitivity threshold and response characteristics of infrared detection in the beetle *Melanophila acuminata*," *Comparative Biochem and Physio*, Part A, 128:805-819, 2001.

Hammer DX, "Infrared Detection in *Melacnophila Acuminata*", PhD Dissertation, The University of Texas, 2001.

Hammer DX, D Davé, TE Milner, B Choi, HG Rylander III, AJ Welch, "Investigation of the trandsuction mechanism of infrared detection in *Melanophila:* photo-thermal-mechanical hypothesis," *Comparative Biochem and Physiol*, Part A, 132:381-382 2002.

Hammer DK, J Seigert, MO Stone, HG Rylander III, AJ Welch, "Infrared spectral sensitivity of *Melanophila acuminate*," *J Insect Physiology*, 47:1441-1450,2001.

Seigert, J, "Exploration into the transduction mechanism of the infrared sensitive pit organ of *Melanophila acuminata*: Optical Low Coherence Reflectometry (OLCR) study, MS Thesis, The University of Texas at Austin, Austin, TX, 2001.

B. Dr. Motamedi (UTMB):

1. Publications

Dr. Motamedi	Total
Refereed scientific/technical journals	4
Peer scientific/technical journals	0
Generic scientific/technical journals	0
Generic magazines	0
Scientific/technical books	0
Other book	0
Invited society-level presentations	0
Invited society-level session chair	0
Other society-level presentations	4
Other invited presentations, conferences, sessions, etc.	0
Other presentations, conferences, sessions, etc.	1
Patents and patent disclosures	0
Unpatented inventions	0
Other (identify)	0

2. Scientific and/or technical accomplishments

i. Accomplishment

Receptors located in the facial pit organ of certain species of snake signal the presence of prey. These receptors are believed to be activated specifically by infrared radiation suggesting that they may be low threshold temperature receptors. However, whether the mechanism of signal transduction in these receptors requires a photosensitive protein similar to that found in photoreceptors, or whether they are specialized receptors that sense prey temperature is unclear.

At the tissue level, we have recorded in vivo from the nerve innervating the pit organ of snakes belonging to the family of *Crotalinae* while stimulating the receptive area with well defined optical stimuli. The objective was to determine the sensitivity of these receptors to a wide range (0.400 – 10.6 μm) of optical stimuli to determine if signal transduction was initiated by a temperature-sensitive or photo-sensitive protein. We have found that receptors in the pit organ exhibited a unique broad response to a wide range of electromagnetic radiation ranging from the near UV to the infrared.

Significance. The spectral tuning of these receptors in response to discrete frequencies spanning the UV to the IR parallels exactly the absorption spectra of water and oxyhemoglobin, the predominant chromophore in tissue, to this same radiation, supporting the hypothesis that these are receptors activated by a minute change in temperature induced by direct absorption of optical radiation in the thin membrane of pit organ, a photo-thermal interaction that can induce a highly localized

temperature changes in tissue during exposure of pit organ to visible and infrared radiation.

ii. Accomplishment

At thecellular level, we have demonstrated that neurons cultured from the trigeminal ganglia innervating the pit organs have two unique temperature-activated cation currents. Whole cell voltage-clamp recordings indicate that a population of neurons show an inward current (I_{heat}) when heated from room temperature, resulting in depolarization of the neuron. Cooling from room temperature resulted in an outward current, suggesting that the temperature-activated current is on at relatively low temperatures. Ion substitution and Ca^{2+} imaging experiments indicate that the heat-activated current is primarily a monovalent cation current. Another small population of neurons responded to cooling with a unique transiently-activated inward current (I_{cool}).

Significance. Our findings indicate that the molecular mechanisms of highly sensitive heat detection in snakes could involve multiple temperature-sensitive ion channels having distinct ion selectivity and temperature activation characteristics.

3. Scientific and technology transitions
   a. IR Parameters
   There were significant amount of scientific transition between the investigators at UTMB and other members of MURI team. In particular, our group worked very closely with Drs. Welch, Pearce and Rylander and Dan Hammer at UT Austin to develop a better understanding of the electrophysiological response of snake and beetle to IR radiation as well as providing the experimental results that were used to develop mathematical model simulating IR receptor in the snake. We have also shared our findings with investigators at Wright-Patterson AFB which should provide an opportunity to closely collaborate with this group on the development of new concepts for identification of proteins and incorporation of snake heat receptors in new thermal sensors.

4. Other

   a. Publications

   Moiseenkova V Yu, BA Bell, M Motamed, E Wozniak, BN Christensen, "Wide-band spectral tuning of heat receptors in pit organ of the copperhead snake *(Crotalinae)*, *Amer J Physiol, Regul Integr Comp Physiol*, 284:R598-R606, 2003.

   b. Training activities

Vera Moissenkova is a graduate student who is working toward her PhD in the Department of Physiology with a concentration in Bioengineering at th University of Texas Medical Branch. Her research was supported by MURI and has received considerable recognition.

C. Dr. John Pearce

1. Publications

Dr. Pearce	Total
Refereed scientific/technical journals	0
Peer scientific/technical journals	3
Generic scientific/technical journals	0
Generic magazines	0
Scientific/technical books	0
Other book	0
Invited society-level presentations	0
Invited society-level session chair	0
Other society-level presentations	0
Other invited presentations, conferences, sessions, etc.	0
Other presentations, conferences, sessions, etc.	0
Patents and patent disclosures	0
Unpatented inventions	0
Other (identify) PhD	1

2. Scientific and/or technical accomplishments

i. Accomplishment:

A calibrated black body source was designed and constructed for the electro-physiologic experiments conducted on the pit organ in pit vipers by the team at the University of Texas Medical Branch in Galveston (UTMB). The black body source was calibrated and its illumination uniformity tested using existing thermal imaging equipment at UT Austin. An adjustable aperture was added to the output plane of the black body by UTMB investigators.

Significance. The calibrated source was employed in electro-physiology experiments at UTMB.

ii. Acomplishment

Inspection of the structure of the pit organ suggested that it may function as a bolometer: all incoming infrared (IR) thermal radiation absorbed by the pit membrane was converted into a neural signal in the trigeminal nerve. If so, the nerve impulse firing rate would be proportional to the total IR received power. The purpose of this phase of the investigation was to determine whether the IR reception was bolometric (i.e. by heat transfer) or photonic (i.e. by means of an IR specific protein).

Two Finite Difference Method (FDM) numerical models of the pit organ and sensing membrane were generated, tested and applied. The first model was an IR optical model

that calculated the 2-D pit sensing membrane IR illumination pattern from a specified target. The target could be elliptical or rectangular of arbitrary dimension (2-D) at a specified temperature and specified range and viewing angle from the pit organ. The organ cavity was modeled as a 3-D ellipsoid with specified major and minor axes in all three dimensions and specified depth from the surface. The membrane was modeled as a 2-D ellipse suspended at a specified location in the pit ellipsoid. Dimensions were taken from Transmission Electron Micrographs, as given by Dr.. Anke Schmitz of The University of Bonn. See Figure 1 for the model space used in the calculations.

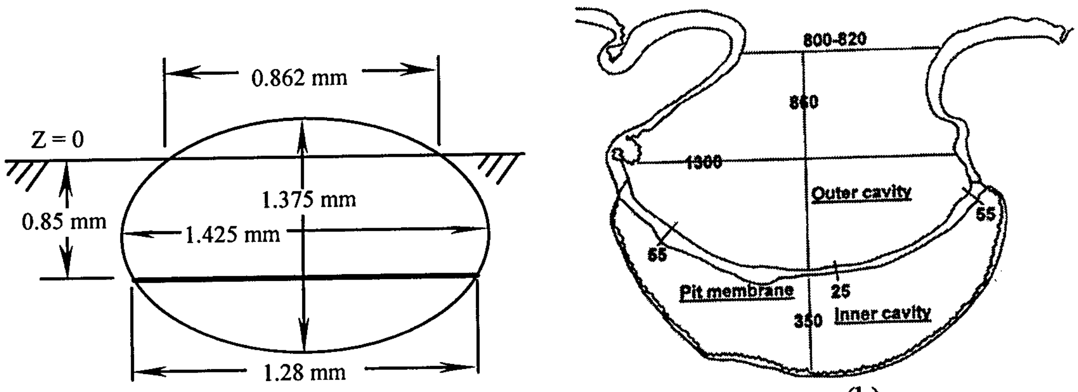

Figure 1: Pit organ geometric model. a) 3-D ellipsoid located as shown with surface aperture (0.862 mm) and sensing membrane (1.28 mm) determined by its depth and minor axis deimension. b) drawing made from original TEM data provided by Dr. A. Schmitz (Bonn)

The second FDM model accepted the IR illumination function from the first model as input for a transient thermal model of the membrane thermal response. The membrane is extremely thin and the IR illumination is of very low intensity. The resulting time step was on the order of 3 μs, and the temperature rise was so small that double precision arithmetic wazs required to prevent the signal from disappearing into the arithmetic uncertainty of the computer. Both models were executed on a Dual G4 500 MHz computer. The optical model solved in 10 minutes and the thermal model typically required 30 minutes to solve. The membrane field of view has a full-width-half-max half-angle of 25 degrees.

Significance. Electrophysiologic data on the power spectral density of the neural impulses recorded from the trigeminal nerve were provided by the UTMB team. The experimental results were compared to numerical model results of the specific experiments. The numerical model results agreed with the electro-physiologic experiments when the "reference" temperature was the pit membrane temperature, 33 °C in the experiments, not the room temperature, 23 °C (see Figure 2). This result confirms that the operating principle of the membrane is bolometric rather than photonic.

The temporal response (Figure 3) indicates the extreme sensitivity of the pit membrane structure. In the model the target was the black body at 40 °C and a range of 10 cm (predicted total membrane received power about 3 μW).

3. Scientific and technology transitions
   a. Model Parameters
   (i) Received electrophysiological data from:
       B. Christensen, V. Moiseenkova and M. Motamedi (UTMB)

   (ii) Received pit cavity and membrane morphologic data from:
       A. Schmitz, (Institut fur Zoologie)

   (iii)Used optical properties of corneum provided by:
       M. Stone (Hardened Materials Branch)

   (iv)Used thermal conductivity of membrane corneum provided by:
       V. Tsukruk (ISU)

4. Other

Publications

Pearce JA, "Numerical models of pit viper IR sense organs," *Proc 18t Ann Conf Houston Soc for Engr in Med and Biol*, February 2000.

Pearce JA and A Schmitz, "Pit viper infrared sense organ," *Proc 2000 World Cong on Med Physics and Biomedical Engr,* Chicago, July 2000.

Pearce JA and A Schmitz, "Numerical models of viper IR pit organs," ASME *Int Cong and Expos on Mech Engr*, New York, November 2001.

Protsenko, Dmitry, "Electrosurgical tissue resection: a numerical study," PhD Dissertation, The University of Texas at Austin, 2002.

Training activities

Dmitry Prosenko's research was supported by the MURI.

D. Dr. Dean Neikirk:

1. Publications

Dr. Neikirk	Total
Refereed scientific/technical journals	0
Peer scientific/technical journals	1
Generic scientific/technical journals	0
Generic magazines	0
Scientific/technical books	1
Other book	0
Invited society-level presentations	0
Invited society-level session chair	0
Other society-level presentations	0
Other invited presentations, conferences, sessions, etc.	0
Other presentations, conferences, sessions, etc.	0
Patents and patent disclosures	0
Unpatented inventions	0
Other (identify)	0

2. Scientific and/or technical accomplishments
   a. Micromachining
   (i) Accomplishment:
   Overall, Neikirk's group is attempting to translate the information garnered from the study of biological IR detection into artificially constructed systems. Neikirk's work is concentrating on the "structural" aspects of IR detection, making use of IC processing techniques to build optical and thermal structures via "micromachining." Several of the structures already identified in biological IR detection fall into this area. Neikirk is using micromachining to fabricate artificial structures with enhanced, selective wavelength IR absorption via interference effects. To investigate the possibility of producing "color-vision" IR focal plane arrays, micromachined IR wavelength selective microbolometers have been fabricated, characterized and modeled. Using micromachining, the bolometer is supported by long and narrow suspension legs to increase the thermal impedance. Constructive interference produced by a mirror placed a quarter wavelength of the incoming infrared signal behind the microbolometers is used to enhance the absorption of the microbolometer at specific infrared wavelengths. Wavelength dependent power coupling has been verified using infrared measurements.

   (ii) Accomplishment:
   Construction of thermally isolated bolometric platforms using silicon micromachining. Investigation of various "tether" arrangements and influence on mechanical, thermal performance.

iii. Accomplishment:
Construction of resonant cavity structures (based on micromachined Fabry-Perot structures) to enhance and tune IR absorption of bolometer elements.

iv. Accomplishment:
Measurements of thermal impedance, responsivity, and infrared spectral response.

v. Our most significant accomplishment during the last year has been the fabrication and testing of an actual wavelength selective infrared microbolometer detector. This has demonstrated the feasibility of integrating "color discrimination" into room temperature infrared detectors.

Significance: Accomplishments (i) – (v): Our most significant accomplishment was the fabrication and testing of an actual wavelength selective infrared microbolometer detector. This has demonstrated the feasibility of integrating “color discrimination” into room temperature infrared detectors.

3. Scientific and technology transitions

There are currently a large number of optical sensors whose spectral ranges span from the x-ray range to the far infrared region. In the visible region, silicon semiconductor systems dominate the sensor field. For example, Charge Coupled Devices (CCDs) are now available commercially which exhibit extraordinarily high sensitivity values and large dynamic ranges. When combined with color selective filters, these CCD systems exhibit spectral response features similar to those exhibited by the human eye. Unfortunately, the band gap limitation of silicon precludes its use for wavelengths greater than ~1.1 μm. For a number of important imaging, remote sensing, surveillance, multi- and hyper-spectral applications, it would be desirable to create sensor arrays which display the capacity to respond to infrared wavelengths. Indeed, the infrared region provides a vast amount of information regarding the chemical composition of the absorbing media. Nearly all organic compounds and most inorganic compound exhibit species specific IR-based absorbances that are routinely exploited for their identification and quantification. Thus, the development of a multi-spectral capability with functionality in the infrared region would have a profound influence on a number of medical/environmental/processing applications.
In contrast to photon detectors, which are sensitive only to wavelengths shorter than the cutoff, thermal detectors such as a conventional bolometer, are, in principle, sensitive to all wavelengths. The absorption of light raises the temperature of the device and this in turn results in changes in a temperature-dependent parameter such as electrical conductivity. As a consequence, the output of a conventional bolometer is usually proportional to the amount of energy absorbed per unit time by the detector and, provided the absorption efficiency is same for the all wavelengths, is independent of the wavelength of the light. In addition, dark current does not limit thermal detectors. Therefore, they can be operated inexpensively at room temperature. Room temperature microbolometers arrays have become widely available for use in infrared imaging systems. The use of micromachining to reduce thermal mass and increase thermal

resistance, combined with a CMOS compatible process, has allowed the development of sensitive, large pixel count, low cost focal plane imaging arrays. The development of tunable multi-spectral capability with functionality in the infrared region would have a profound influence on a number of military/civilian applications.

Microbolometer design can be separated into two parts. The first part is optimizing the sensing of resultant temperature, and the other part is the absorption of radiation. In an ideal device, the absorber must provide total absorption of the incoming radiation and furthermore, it must convert the electromagnetic radiation into heat. Our work focused on the design of a structure with an absorbed power coupling efficiency that is wavelength dependent.

Broadband absorption in a thin conducting film is obtained when its sheet resistance is matched to the impedance of free space (377 ohm) (Figure 1). As long as the required conductivity of absorbing material remains unchanged within the operating wavelength region, the impedance matching technique is a valid approach for broadband absorption. The amount of power absorbed, transmitted, and reflected is 4/9, 4/9, and 1/9 of the incoming incident power, respectively. Thus 44 percent of the incident power is absorbed without explicit wavelength dependence.

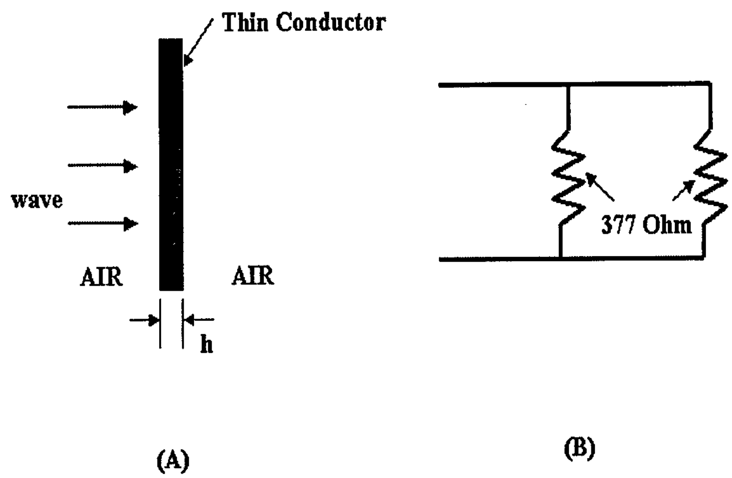

Figure 1. Schematic view of matching a radiation absorber to free space; (A). Geometry (B) Equivalent Circuit.

Enhanced absorption can be increased by placing a reflecting short at one-quarter wavelength behind the absorbing layer, but would make the absorber wavelength sensitive. Figure 2 shows the modified configuration of matching impedance to free space shown in figure 1. Theoretically, 100% radiation absorption in a thin film can be achieved at the resonant point with a perfect mirror placed [(odd integer)/4]* λ behind the absorbing layer. This is sometimes referred to as "space cloth," or when used with a microbolometers, as a Fabry-Perot tuned microbolometers. The mirror layer must be a perfect mirror, i.e., must use a material with a high conductivity. For example, gold is a good candidate as a mirror material due to high conductivity and high reflectivity at infrared wavelengths. Figure 3 illustrates the micromachined structure used to test the performance of such a wavelength selective microbolometer.

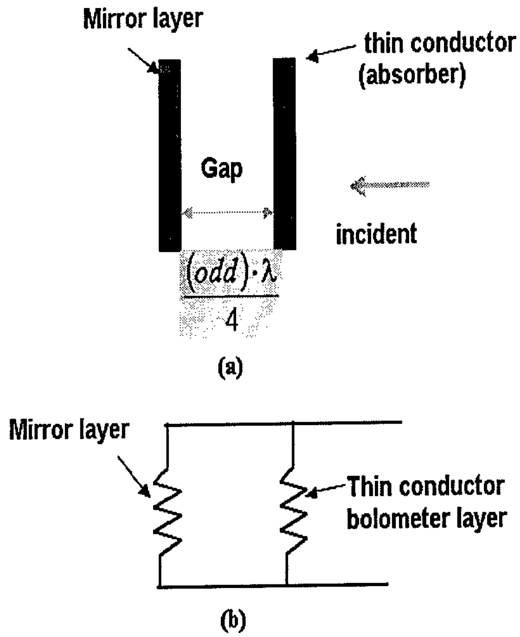

Figure 2. Schematic view of enhanced radiation absorption in a thin film with mirror placed [(odd integer)/4]* λ behind absorbing layer. (a) Geometry and (b) Equivalent circuit.

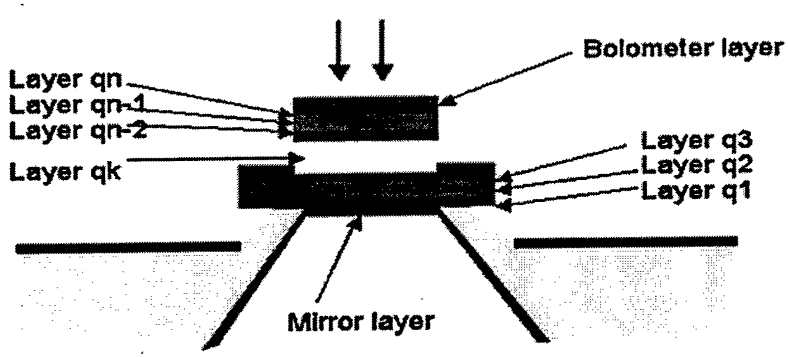

Figure 3: Schematic view of a micromachined wavelength selective microbolometer.

Assuming that the power spectral density is constant, the power coupling spectral response for different air gaps is shown in Figure 4. In addition, we have fabricated several prototypical devices and measured their infrared response. The results of these measurements are also indicated in Figure 4, showing good agreement between the calculated and actual response of the device.

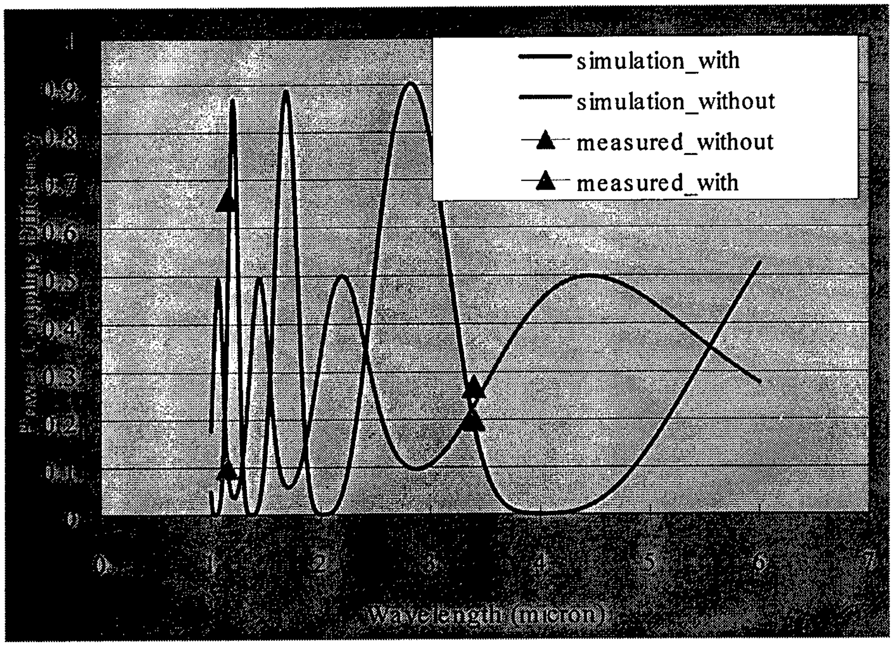

Figure 4: Micromachined microbolometers for “color vision” in the infrared: calculated and measured response of the structure schematically illustrated in Figure 3. Two devices are shown: one with, and one without, a mirror to enhance IR absorption.

4. Other

Publications

Eames SJ, S-J Yoo, J. C. Warner, D. P. Neikirk, and J. T. McDevitt, “Lithographically patterned Superconductor Bolometer Detectors for Visible and Near-Infrared Radiation incorporating Wavelength Selective Light Absorbing Elements,” presented at SPIE Symposium 3790: Engineered Nanostructural Films and Materials, Denver, CO, July 23, 1999, pp. 160-168.

Yoo, Seung-Jin, “Micromachined Wavelength Selective Microbolometer Sensors Operating at Room Temperature” PhD Dissertation, The University of Texas at Austin, December 2000.

Training Activities

PhD student Seung-JinYoo was supported by this MURI project.

E. Dr. Vladimir Tsukruk (ISU)

1. Publications

Dr. Tsukruk	**Total**
Refereed scientific/technical journals	12
Peer scientific/technical journals	1
Generic scientific/technical journals	0
Generic magazines	0
Scientific/technical books	0
Other book	0
Invited society-level presentations	3
Invited society-level session chair	6
Other society-level presentations	7
Other invited presentations, conferences, sessions, etc.	7
Other presentations, conferences, sessions, etc.	4
Patents and patent disclosures	0
Unpatented inventions	0
Other (identify)	0

2. Scientific and/or technical accomplishments
   a. Material Properties
   (i) Accomplishment:
   Mapping the surface morphology and distribution of micromechanical and microthermal properties in biological receptors with a submicron resolution was accomplished. Morphology of snake pits and non-specific scales were studied and specific organized micropit and microfibrillar morphology was studied in detail.

   Significance:
   For the first time, data on surface microstructure were obtained for *living tissue* under close to *in-vivo conditions*. Technique for pit sample mounting and sectioning for SPM studies was developed. Sections at different depths were studied and a complicated system of highly branched neuron nanofibrils was observed. Scanning thermal imaging revealed highly inhomogeneous distribution of heat dissipation in pit areas. Micromechanical studies revealed much more compliant surface in pit areas as compared to between-pit skin. Analysis of combined micromechanical and microthermal data allowed to conclude photothermal mechanism of thermal sensing in snakes. A model of artificial IR sensor (thermo-optial cavity) based on bio-inspired principles was proposed.

   (ii) Development of the microthermal imaging mode and testing its applicability to snake skins was undertaken. This included the development of probing protocol for microthermal measurements and calibration procedure, identification of lateral resolution of thermal probes, and microthermal imaging of pit areas.

Significance:
Data processing for elastic modulus and adhesive forces surface histogram calculations was developed concurrently with microthermal probing. Micro-thermo-mechanical studies of beetle IR detectors involved measuring local stiffness, microstructure, and surface properties of microtomed samples, high-resolution investigation of the multilayer geometry at various temperatures, and studying thermo-micro-mechanical properties. Microlayered structure of beetle IR detectors was shown is related to variable shearing properties. Thermal expansion coefficient measured for multilayered structure of beetle IR detectors was close to one speculated from thermo-mechanical hypothesis of beetle IR imaging.

3. Scientific and technology transitions
    a. Micromechanical Mapping

Software package has been developed with partials AFOSR support for micromechanical mapping of compliant materials. Paper with complete description is under preparation and software will be made available to SPM community as "freeware" through the PI website when brief manual will be finished.

4. Other

Publications

Gorbunov V, N Fuchigami, M Stone, M Grace, VV Tsukruk, "Biological thermal detection. II: Micromechanical and microthermal properties of snake infrared receptor organs," *Biomacromolecules*, 2001, accepted

Fuchigami N, J Hazel, VV Gorbunov, M Stone, M Grace, VV Tsukruk, "Biological thermal detection. I: Ultra-microstructure of pit organs in infra-red imaging snakes," *Biomacromolecules, 2,* 757, 2001.

Hazel J, N Fuchigami, V Gorbunov, H Schmitz, M Stone, VV Tsukruk, "Ultra-microstructure and microthermomechanics of biological IR detectors: materials properties from biomimetic prospective," *Biomacromolecules, 2*, 304, 2001.

Tsukruk VV, I Luzinov, K Larson, S Li, DV McGrath, "Intralayer reorganization of photochromic molecular films," *J. Mater. Sci. Lett.*, *20*, 873, 2001

Tsukruk VV, Z Huang, "Micro-thermomechanical Properties of Heterogeneous Polymer Films," *Polymer*, *41*, 5541, 2000.

Tsukruk VV, VV Gorbunov, Z Huang, SA Chizhik, "Dynamic Microprobing Of Viscoelastic Polymer Properties," *Polymer Intern. 49,* 441, 2000.

Gorbunov VV, N Fuchigami, VV Tsukruk, "Microthermal Analysis With Scanning Thermal Microscopy. I. Methodology and Experimental," *Probe Microscopy*, *2*, 53, 2000.

Gorbunov VV, N Fuchigami, VV Tsukruk, "Microthermal Analysis With Scanning Thermal Microcopy. II: Calibration, Modeling, and Interpretation," *Probe Microscopy, 2,* 65, 2000.

Sidorenko, C Houphouet-Boigny, O Villavicencio, M Hashemzadeh, DV McGrath, VV Tsukruk, "Photoresponsive Langmuir Monolayers From Azobenzene-Containing Dendrons," *Langmuir, 16,* 10569, 2000.

Gorbunov VV, N Fuchigami, I Luzinov, VV Tsukruk, "Microthermal Probing Of Ultrathin Polymer Films," *High Performance Polymers, 12*, 603, 2000.

Hazel J, M Stone, MS Grace, VV Tsukruk, "Nanoscale Design Of Snake Skin For Reptation Locomotions Via Friction Anisotropy," *J. Biomechanics, 32,* 477, 1999.

Gorbunov VV, N Fuchigami, JL Hazel, VV Tsukruk, "Probing Surface Microthermal Properties By Scanning Thermal Microscopy," *Langmuir, 15*, 8340, 1999.

Tsukruk VV, VV Gorbunov, N Fuchigami, "Microthermal Analysis of Polymeric Materials," *Thermochimica Acta* 2003, 395, 151.

Gorbunov V, N Fuchigami, M Stone, M Grace, VV Tsukruk, "Biological Thermal Detection: Micromechanical and microthermal properties of biological infrared receptors," *Bimacromolecules,* 2002, 3, 106.

F. Dr. John McDevitt:

1. Publications

Dr. McDevitt	Total
Refereed scientific/technical journals	8
Peer scientific/technical journals	0
Generic scientific/technical journals	0
Generic magazines	2
Scientific/technical books	0
Other book	0
Invited society-level presentations	21
Invited society-level session chair	3
Other society-level presentations	0
Other invited presentations, conferences, sessions, etc.	0
Other presentations, conferences, sessions, etc.	0
Patents and patent disclosures	0
Unpatented inventions	0
Other (identify)	0

2. Scientific and/or technical accomplishments

a. Multiple Wavelength Detectors

(i) Accomplishment:

Improved device characteristics: After developing the dye doped polymer/YBCO-based sensing devices which functioned as color sensor, we have begun in the past year to improve on both the sensitivity of the devices as well as their wavelength range. The sensitivity of such devices can be improved by lengthening the microbridge geometry. To this end, a lithographic mask incorporating a 2x2 meandering path microbridge array has been created. Each microbridge can be addressed separately and functions as a single "cone" when combined with a polymer layer doped with a dye.

Significance: The usable wavelength range of the dye in polymer / YBCO sensor has been extended by the identification of suitable dyes absorbing in the NIR. A sensor that detects green, red and two bands of NIR light has been created. The identification of other NIR absorbing candidates is expected in the near future.

ii. Accomplishment:

Switchable color sensors demonstrated.

Significance: Additional work has been completed in the area of switchable color sensors. A system based on a photochromic dye has been fashioned. The system can be repeatably switched many times. An alternative switchable device has been created using electrochromic conducting polymers. These may be either grown onto the surface of the YBCO by electrochemical methods, or if soluble, deposited by means

of spray coating. The color change is achieved by doping the polymer using chemical or electrochemical methods. Several systems with interesting color changes have been identified and will be more fully investigated in the next year.

iii. Accomplishment:
Wavelength tunable structures from AU colloids: The synthesis of colloidal gold (~6 nm diameter) and colloidal silver (~13 nm) in non-aqueous media has been accomplished following literature procedures. Characterization of the colloidal suspensions was done by UV-Vis spectroscopy and by TEM. Furthermore, other methods of surface attachment are being examined. These include nanoparticle attachment via bi-functional molecules that crosslink particles. Thus, the particles fall out of solution onto the surface and form a pseudo-hexagonal array. Also, molecules like 4-aminothiophenol has been used to create layers of gold nanoparticles. PAMAM Dendrimers were used as nanoreactors, i.e. nanoparticles can be grown inside the dendrimer cavity and the cavity determines the size of the particle. Dendrimers with copper and nickel nanoparticles were grown.

Significance: Dendrimers with active amine groups on the outside will be anchored to YBCO and then Cu, Ag, or Au nanoparticles grown inside for wavelength selectivity.

iv. Accomplishment:
Wavelength tunable structures from conductive polymers: Poly(3-hexylthiophene) [PHT] was spray coated from a solution of toluene onto a YBCO microbridge.

Significance: By chemical doping of the PHT with AuCl3 we have produced a switchable device. The undoped form absorbs light in the visible and the doped form absorbs light in the far visible/near infrared. Switching can be repeated many times. Further experiments are presently being done to determine the optimal thickness of the PHT layer.

3. Scientific and technology transitions
    a. Chemistry
        Provided IR dyes to Dean Neikirk

Recent experiments in the McDevitt laboratory have shown that sensitive bolometric platforms can be sensitized to respond selectively to specific wavelength ranges in the infrared. The initial demonstration of this "IR cone" concept will be expanded in future studies in an attempt to create a new generation of multispectral analyzes which exhibit enhanced performance characteristics.

G. Dr. Hagan Bayley (TAMU):

1. Publications

Dr. Bayley	Total
Refereed scientific/technical journals	3
Peer scientific/technical journals	0
Generic scientific/technical journals	4
Generic magazines	0
Scientific/technical books	0
Other book	0
Invited society-level presentations	9
Invited society-level session chair	0
Other society-level presentations	9
Other invited presentations, conferences, sessions, etc.	0
Other presentations, conferences, sessions, etc.	0
Patents and patent disclosures	1
Unpatented inventions	0
Other (identify)	0

2. Scientific and/or technical accomplishments

a. Protein Engineering

i. Accomplishment:

Engineered a pore that contains a polymer within a cavity in the lumen. This difficult piece of protein engineering was published in J. Am. Chem. Soc. and was a necessary precursor to the placement of responsive polymers within the α-hemolysin pore. The work was mentioned in Science magazine. An additional paper has now been published in *J. Gen. Physiol.* and this was the subject of a special editorial.

Significance: We are sequentially achieving the specific aims stated in the proposal and the progress reports. The significance of these aims should be apparent as they encompass the goals of the project.

ii. Accomplishment:

Placed responsive polymers in the cavity of the α-hemolysin pore and demonstrated that the modified pore can function as a sensor element. We showed that a protein sensor can be made by our polymer technology. The work was featured on the cover of Nature Biotechnology and was the subject of a News & Views in the same issue.

Significance: There have been spin-offs, such as the ability to detect proteins by stochastic sensing. (See the *Nature Biotechnology* article).

(i) Accomplishment:

Obtained pores with large polypeptide inserts within the cavity. This is the

groundwork required for placing temperature-sensitive polymers within the α-hemolysin pore. It is unpublished, but described in our MURI presentation (see ppt file).

Significance: This work has been judged significant by others, *i.e.* the two editorials and the cover of *Nature Biotechnology.*

3. Scientific and technology transitions

Investigation of the mechanical properties of protein pores with Dr. Vladimir Tsukruk (ISU)

H. Dr. Michael Grace (FIT):

1. Publications

Dr. Grace	Total
Refereed scientific/technical journals	11
Peer scientific/technical journals	0
Generic scientific/technical journals	4
Generic magazines	0
Scientific/technical books	1
Other book	0
Invited society-level presentations	1
Invited society-level session chair	0
Other society-level presentations	9
Other invited presentations, conferences, sessions, etc.	0
Other presentations, conferences, sessions, etc.	0
Patents and patent disclosures	1
Unpatented inventions	0
Other (identify) PhD/MS	1/2

1. Biochemistry of IR receptor neurons in pit organ and TGG:

Understanding the transduction mechanism of infrared receptors in the snake pit organ will allow an understanding of the high sensitivity of the system. A fundamental unanswered question is whether IR transduction is based upon a thermal (change in molecular momentum) or a photic (photoisomerization of a molecular bond) mechanism. Many (if not most or all) vertebrate photoreceptors utilize opsin-based photopigments. These photopigments vary considerably across species and from cell type to cell type in their photic responsiveness or action spectrum; some known opsins provide efficient transduction of near infrared radiation.

We showed that IR receptor terminals in the pit organ are immunocytochemically distinct from retinal photoreceptors based upon opsin content. A battery of anti-opsin antisera, most of which immunocytochemically labeled retinal photoreceptors in *Python*, all failed to label IR terminals of the pit organ. Because these antisera recognize distinct and highly conserved epitopes of opsin molecules, these results indicate that IR absorption is unlikely to be based upon an opsin-like photopigment.

We also searched the Iinfrared-sensitive neuronal terminals for common components of signal transduction in other systems (components that are common among divers signaling mechanisms). One common component of intracellular neuronal signaling is calcium, which often exerts its effects through calcium-binding proteins. Two calcium-binding proteins, calmodulin and S-100, were immunologically localized to the pit organ terminals. These proteins are the first identified signal transduction components localized to IR terminals. Both are calcium-regulated proteins (as opposed to calcium-sequestering proteins), which likely participate in IR signaling by actively responding to changes in calcium concentration within the terminals upon IR stimulation. Calmodulin was not found in photoreceptors, while S-100 was found in a subset of retinal

photoreceptors. In addition, another calcium-binding protein, calretinin, was localized to photoreceptors but not IR receptor terminals. These results further distinguish IR receptors from opsin-based photoreceptors. In addition, cells of the trigeminal ganglion exhibit immunoreactivity for CaBPs not found in IR-sensitive receptor terminals. These CaBPs include calretinin and calbindin. These soma may represent either interneurons or soma subserving other sensory systems.

We also investigated the roles of vanilloid-receptor like proteins in the infrared system. These represent the best proteinaceous candidates for infrared signal transduction molcules. Our immunocytochemical results show that vanilloid receptor-like proteins exist in infrared-sensitive neurons of the trigeminal ganglion, and suggest that multiple types of vanilliod-like protein may mediate different functions (including both infrared transduction and regulation of absolute sensitivity). Western blots confirm the presence of immunologically reactive proteins of appropriate size.

This work has led to the development of a new collaborative effort aimed at investigating the functional properties of vanilloid-like proteins in isolated neurons from the trigeminal ganglia of infrared-sensitive snakes. In support of this goal, we have developed techniques for isolation and culture of intact trigeminal ganglia and of dispersed trigeminal ganglion cells. These cells are being used in calcium imaging experiments (note role of calcium binding proteins above) aimed at identifying the functional roles of vanilloid receptors. We hypothesize that these proteins are functionally distinct from known vanilloid receptors, and that they may have a much greater sensitivity to incident infrared radiation, or that their spectral sensitivity has been altered through amino acid substitution. Our experiments are directly aimed at these hypotheses.

2. Neuroanatomy of Central IR Information Processing in Snakes

Our work on the central neuroanatomy of *Python*/pitviper IR processing requires detailed knowledge of the general neuroanatomy of the snake brain. While portions of the brains of several different species have been mapped (data published in various papers over the past 30 years), there has never been produced a comprehensive atlas of the brain of any snake species. Therefore, we have produced a two detailed parallel maps of the entire *Python molurus* brain in frontal (coronal) section, one based upon a cellular histological stain and the other based upon a histological stain for fiber tracts. In addition to other nuclei and fiber tracts throughout the brain, we have identified the major known relay points for infrared information. This atlas will be published.

3. Structural Analyses of Pit Organ and IR Receptor Neurons

In collaboration with materials other MURI team members and scientists from MLPJ, Wright-Patterson AFB, we have performed a detailed structural materials analysis of the infrared imaging pit organ, and compared the pit organ with other surface regions including the spectacle of the eye. In collaboration with the Tsukruk group, we have modeled the tribological properties of snake skin and the surface and subsurface structural propoerties of the pit organ that relate to infrared imaging function.

We used scanning probe atomic force microscopy to investigate the microfibrillar nanostructure of the most snake skin in comparison with the ordered array of micropits on the surfaces of imaging organs (pit organ and spectacle). The microfibrillar

arrangement can function to alter adhesive forces between surface epidermis and environmental structures by generating frictional anisotropy. On the other hand, micropit arrays in the surfaces of imaging organs (with distinct spatial arrangements in different imaging organs) may serve as spectral filters to aid in differential spectral tuning of the pit organ and eye. These results are consistent with our earlier observations of pit organ spectral properties (low reflectance and high absorption in the infrared region, particularly at 8-12μm) performed using infrared spectrometry and real-time infrared imaging. These observations are also supported by our analyses using ultra-high resolution scanning probe microscopy. Data from these studies better estimated the dimensions of micropit arrays in living (chemically unaltered) material, and show that the grating spacing should produce efficient reflection of broadband visible light while allowing efficient absorption of infrared radiation.

4. Functional correlates of infrared imaging

A thorough analysis of any complex sensory system must include a detailed understanding of its functional properties in the organism. It is these properties that form a functional framework for the logical design of detailed mechanistic investigations.

We have shown that the snake infrared imaging system operates in concert with the visual system to provide real-time analysis of incident radiation simultaneously in two distinct regions of the electromagnetic spectrum. The brain processing centers utilize visible light and infrared radiation either in concert or alone to form useful spatial images. In effect, snakes seamlessly switch between the two modalities depending upon environmental circumstances.

Wiring of sensory systems is generally thought to be highly plastic, allowing the development of novel wiring diagrams according to sensory experience. We have found that temporary loss of portions of field of view of one or the other system (visual and infrared) after development is compensated for by information from the other system. On the other hand, loss of part of the visual field during development (congenital anophthalmy) produces dramatic effects on wiring of the visual system. However, this same visual loss does not produce a corresponding change in functionality of the infrared system. Thus, the complex mechanisms of neural plasticity in this multi-modal imaging system are partly interdependent, and partly independent, and suggest the importance of maintaining a functional infrared imaging system regardless of the developmental/functional state of the visual system. These results should form the foundation for an effective analysis of complex brain wiring mechanisms and sensory plasticity that will ultimately aid in the development of novel artificial imaging devices.

Finally, we have determined that the infrared imaging systems of crotaline snakes (pit vipers) functions as a thermal contrast detector. We have shown that direction of contrast is unimportant- that effective neural-based targeting occurs in response to both positive and negative thermal differentials, but that targeting effectiveness falls of as thermal differential approaches zero. Though in the living system targeting behavior differs between positive and negative thermal contrast situations, in both cases, the system functions as an equally effective thermal contrast detector. This apparently simple mechanism of contrast detection is likely generated in first and second order neurons easily accessible for further functional analyses.

Publications:

Campbell AL, TA Bunning, MO Stone, D Church, and MS Grace, "Surface ultrastructure of pit organ, spectacle, and non-pit organ epidermis of infrared-imaging boid snakes: A scanning probe and scanning electron microscopy study." *Journal of Structural Biology* 126:105-120, 1999.

Fuchigami N, J Hazel, VV Gorbunov, M Stone, M Grace, and VV Tsukruk, "Biological Thermal Detection I. Ultramicrostructure of pit organs in infrared imaging snakes." *Biomacromolecules* 2:757-764, 2001.

Grace MS, "Behavioral correlates of multi-modal imaging in infrared imaging snakes." 2003 Proceedings: Association of Reptile and Amphibian Veterinarians / American Association of Zoo Veterinarians. (in press)

Grace MS, "Calcium-binding proteins of a novel imaging system: the *Python* infrared-sensing pit organ." *Neuroreport.* (submitted)

Grace MS and SD Roper, "Infrared Sensory Organs." In *Cell Physiology Sourcebook*, 3rd. ed. N. Sperelakis, Academic Press, San Diego, 2001.

Grace MS and OM Woodward, "Altered visual experience and acute visual deprivation affect predatory targeting by infrared-imaging Boid snakes." *Brain Research* 919:250-258, 2001.

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Hazel J, M Stone, MS Grace, and VV Tsukruk, "Nanoscale design of snake skin for reptation locomotions via friction anisotropy." *Journal of Biomechanics* 32:477-484, 1999.

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VanDyke JU and Grace MS, "The role of thermal contrast in infrared imaging by the crotaline snake Agkistrodon contortrix." *Journal of Comparative Physiology.* (in preparation)

Zhu,Y and Grace MS, "Distribution of vanilloid receptor-like proteins in the infrared imaging systems of boid and crotaline snakes." *Journal of Comparative Neurology.* (in preparation)

Training Activities

The following students were all supported in part by MURI funds.

Mr. Shawn Heflick (M.S.)
Mr. Yu Zhu (M.S.)
Mr. Adam Safer (Ph.D. ongoing)
Ms. Atsuko Matsushita (post-doctoral fellow)
Mr. Owen Woodward (B.S.)
Ms. Bree Buck (B.S.)
Mr. James VanDyke (B.S.)